BIBECHANA

A Multidisciplinary Journal of Science, Technology and Mathematics

ISSN 2091-0762 (online)

Journal homepage: http://nepjol.info/index.php/BIBECHANA

Ovarian Histomorphology and Gonadial Cycle of Freshwater Garfish *Xenentodon cancila* (Hemilton-Buchanan) (Beloniformes:Belonidae)

Bharat Raj Subba and Satya Narayan Meheta
Department of Zoology,Post Graduate Campus (TU), Biratnagar, Nepal
email:subbabharat@yahoo.com

Article history: Received 5 December, 2011; Accepted 26 December, 2011

Abstract

The histomorphology and gonadial cycle of a freshwater garfish *Xenentoton cancila* revealed that the ovaries of the fish pass through resting, early maturing advanced maturing ,pre-spawning, spawning and spent phases within one year.The oogenetic activity starts in November and continues upto June(GSI=10.19)-July(GSI=11.38),when ovaries are full of yolky eggs.Only young oogonia and oocytes I appeared in September to October.The oocytes of I,II,III,IV and V stages were present during November to February.The oocytes VI and VII were seen dominated by ripe oocytes during June to late July when spawning takes place.Asychronism mode of oocyte development was recorded in *X.cancila.*

Keywords: *Xenentodon cancila*; oocyte development; reproductive cycle; spawning

1. Introduction

The studies on fish biology encompassing habitat ecology,ovarian morphology,spawning,physiology and reproductive habits have been indespensible for the fish culture development and safeguard of fish species.Pisciculture has been an important adjunct to aquaculture on one hand and the conservation of fish has been a global attention because of climate change and population explosion on the other.Conserving fish is only possible when the life cycle is fully understood. The study of spawning habit of a fish is carried out in the natural habitat but as fish are aquatic ,it is not possible to observe all the steps taking place during spawning and before that too.Therefore, considering the above facts, the study of ovarian histomorphology of the freshwater garfish *Xenentodon cancila* has been made to understand the reproductive potential of the species in the natural habitat.

Several workers have studied the gonads of fishes. Among many, most important contributions on the histological and seasonal changes in the ovary of fishes were made[1-11]. Shrestha and Khanna[12] have worked on structure and histological changes in the ovary of the Nepalese snow trout *Schizothorax. plagiostomus*. . Rita and Nair [13] made studies on oogenesis in a tropical loach, *Lepidocephalus thermalis* and hillstream loach *Noemachelilus triangular.* Toshimori, and Fumioki [14] studied gap junctions between microvilli of an oocyte and follicle cell in teleosts, *Plecoglossus attivelis*. Chakrabarti and Mandal [15] studied seasonal histological changes in the vary of *Puntius japanieus*.

In spite of large contributions noted above, the study in ovarian structure of freshwater garfish (Kauwa) is meager indeed., taking this point in view, Subba [16] has carried out an investigation on ovarian histomorphology and gonadial cycle of a hill-stream fish *Lepidocephalichthys guntea*.

2. Materials and Methods

Live specimens of *Xenentodon cancila* were collected every month from Muriyadhar which runs parallel to east Koshi dam in Sunsari. The weight of the specimens and their ovaries were recorded in situ.The ovaries taken out were washed in distilled water properly so as to remove blood stains. Fresh pieces from anterior, middle and posterior regions of the ovary were taken and fixed separately in Bouin's fixative. Serial sections were cut at 5 to 6μ and stained in iron haematoxylin using eosion as a counter stain. Mallory triple stain was also used to study connective tissues. The gonadosomatic index (GSI) was calculated for every month as below.

$$\text{GSI} = \frac{\text{Weight of the ovary}}{\text{Total weigh of the fish}} \times 100$$

3. Results

3.1 Ovarian Morphology

The ovaries of ***Xenentodon cancila*** **are** paired and elongated sac like structures which lie in the abdominal cavity ventral to the kidney. They are attached to the body wall by means of the mesovarium. The anterior portions of the ovary are free, broad but posterior ends are united into one to form oviduct (Fig 1).

The ovary is of typical teleostean type consisting of an ovarian wall and ovocoel. The ovary is covered with a thin peritoneal covering or serosa. Beneath this is the elastic and muscular connective tissue, The connective tissue fibers bear the blood vessels, project into the ovarian lumen, ovocoel to form finger like folds known as the ovigerous lamellae, which encloses numerous ova in different stages of development and growth. The ovaries are of light cream colour during early stages of maturation but become yellow in the breeding season, ripe ova project out of the surface and gonads become semitransparent.

As maturity advances the weight of the ovaries also increases gradually and reaches maximum in the mouths of Jun-July showing highest GSI value was 11.3772. The weight became minimum in the months of Oct-November with GSI 0.9416. After spawning the ovaries became, thin flaccid, and were considerably reduced in size.

3.2. Histology

The wall of the ovary is composed of an outer thin layer, peritoneal membrane, which is the outermost layer, overlying the tunica albuginea. The tunica albuginea is made of fibrous connective tissue and blood capillaries. The inner most layer is the germinal epithelium which projects into the ovocoel in the form of lamellae. These ovigerous lamellae are the seat for the development of oogonia, which are visible in various stages of development. Oogonia of various stages are arranged on either side of these lamellae. These oogonia mature into oocytes.

4. Developing Oocytes

An oogonium has a large nucleus and a thin layer of ooplasm which is chromophobic. Each oogonium undergoes successive maturation divisions and form new generation of oocytes. Before ripening an ovum, a series of cytonuclear changes take place in the oocyte.

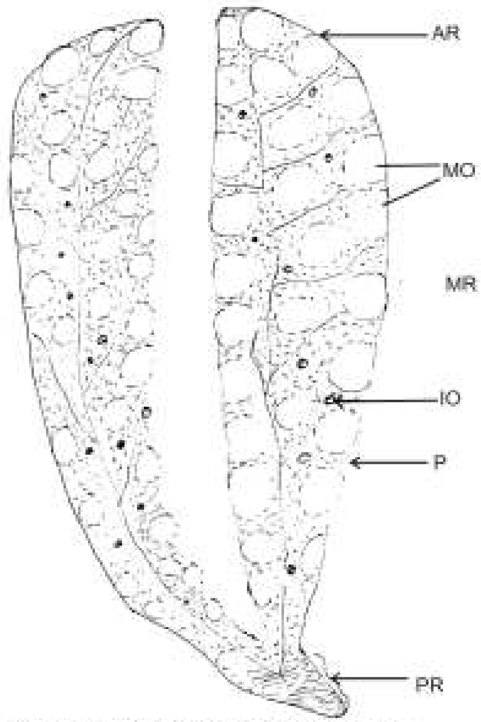

Fig. 1. General morphology of ovary of *Xenentodon cancila*
(AR = Anterior region, MO = Mature oocytes, MR = Middle region, IO= Immature oocytes, P = Peritoneum, PR = Posterior region.)

Stage - Oocyte (Plate-I a,Plate-2 g) :The earliest stage of germ cell in the ovary is the oogonium, which arises from the residual oogonia, occurring in the year. An oogonium has a large nucleus which is single thin layer of ooplasm which does not take basic stain. This stage is also known as chromatin nucleus stage. Each oogonium passess through seven different stages called the oocytes, before it becomes a ripe ovum. The following stages of oocytes have been identified in the ovary of this fish. The mean diameter of an oogonium is 0.035 mm.

Stage - I Oocyte (Plate I a,b Plate-2): In this stage, the oogonia which acquire larger amount of cytoplasm are called oocytes. An oocyte can be distinguished from an oognium because of a prominent round central nucleus and two or three nucleoli. In this stage, oocyte is encircled by an incomplete layer of simple squamous cells. This is the beginning of the follicular layer. The cytoplasm is basophilic. The oocyte in this stage measured 0.1 mm in diameter.

Stage - II Oocyte (Plate I b,d Plate-3): With an increased quantity of cytoplasm, increases strong affinity for basic dyes. In this stage many oocytes have a yolk nucleus situated close to the nuclear membrane in the cytoplasm. The yolk nucleolus ,first appears near the nuclear membrane but moves towards the periphery in the later stage. Several nucleoli of various sizes are arranged along the periphery of nuclear membrane. This stage is conventionally known as perinucleolus stage. The oocyte measured 0.15 mm.

Stage - III Oocyte (Plate Ic, Plate-2 i) :Further development of the oocytes is marked by the formation of a thin layer of follicular cells around the cytoplasm. Owing to the differential affinity to the basic dyes, the zonation is exhibited in ooplasm. The oocytes of this stage show an intensely stained inner zone and lightly stained outer portion. A few nucleoli extrude out of the nuclear membrane into the cytoplasm, some of them can be seen emerging through nuclear membrane while others are found in cytoplasm. Yolk nucleous is seen frequently at this stage. The oocyte, at this stage was of 0.24 mm in diameter.

Stage - IV Oocyte (Plate 1 b,): This stage is characterized by the appearance of minute vaculoes in the ooplasm. The vacuoles appear as if they are empty because they don't take basic dyes. The ocyte is now known to have reached the yolk vesicles stage. As oocytes grow further, more yolk vesicles appear at the periphery and proceed inwards towards the nucleous and fill the entire cytoplasm. At this stage a few of the nucleoli can be seen to pass out of the undulating nuclear membrane. In this stage a vitelline membrane or zona radiata is also recognizable between ooplasm and follicular layer. The oocyte in this stage measured 0.29 mm in diameter.

Stage - V Oocyte (Plate 1 d) : After the cytoplasm has become full of yolk vesicles, yolk begins to appear in the form of small granules in the extra vesicular ooplasm. Most of them are present near the periphery of the oocyte and accumulate in high number. The yolk granules then proceed centripetally to the entire cytoplasm and become impregnated with them. The smaller granules fuse each other in later stage to produce large yolk globules. The process of nuclear extrusion contiunes in some of the oocytes. The zona radiata and follicular layer become more thicker in this stage. The oocytes measured 0.33 mm in diameter at this stage.

Stage - VI Oocyte (Plate 1 e) :This stage is characterized by the appearance of the yolk globules. The nucleus disappears. The yolk starts depositing in the form of large globules in the extravesicular ooplasm. The entire cytoplasm becomes impregnated with yolk globules or granules proceeded in words. Some yolk vesicles of smaller size collect along the periphery of the oocyte and form individual so called cortical alveoli. Outside the follicular layer, a thin layer of fibroblast develop. The oocytes measured 0.44 mm in diameter.

Stage - VII Oocyte (Plate 1f) :At this stage nucleus becomes irregular in outline and smaller in size. The nucleus can be observed gradually migrating towards the periphery of the oocyte. So this stage is known as migratory nucleus stage. The nuclear membrane becomes indistinct and probably after that the contents of the nucleus mingle with cytoplams. The oocyte of this stage measured 0.48 mm in diameter.

Ripe Oocyte: The ripe oocyte is recognized by its largest size, yellowish colour and translucent. Large amount of yolk globules and yolk vesicles may lie scattered in it. It is covered over by three distinguishable layers namely theca an external layer, followed by the follicular epithelium (zona granulosa), and the innermost layer ,zona radiata. Zona granulosa is made up of follicular epithelium. The ripe oocyte is full of yolk and nucleus is not visible. The ripe, oocyte measured 0.56 mm in diameter. This is the largest size oocyte and the ovary contains several ripe eggs. Spawing takes place at this stage. A ripe egg is full of large amount of noncontiguous yolk and numerous yolk vesicles remain scattered in it.

4. Reproduction Cycle

On the basis of histomorphological changes, the ovarian cycle of *Xenentodon cancila* has been divided into the following phases.

Resting phase (October to November) :It extends from October to November. In this phase ovaries are thin translucent, pale and dirty brown in colour, with less vascular supply.

Histologically, the ovary shows ovigerous lamellae, packed with oogonia. The oogonia are budded off from the germinal epithelium and are arranged in nests.

Early maturing phase (December to January) Plate 1 a,b : This phase extends from December to January. During this phase the ovaries become slightly thicker, opaque, yellowish in colour.

Vascularisation is feeble. The ovigerous lamellae are greatly swollen and laden with oocytes of different stages. Tunica albuginea becomes slightly thinner than in previous months. There is an increase in the weight to the ovary.

Advanced maturing phase (February to March) Plate 1 e,f : This phase extends from February to March. In this phase, colour of the ovaries changes into deep yellow. The blood capillaries become inconspicuous because of profuse vascular supply. Number of immature oocytes decreases. The ova are tightly held and the ovary can not be striped by applying gentle pressure. The tunica albuginea becomes extremely thin. Due to the presence of large number of fully mature ova. The ovocoel is greatly reduced. Migratory nucleus reduced in size is seen towards the periphery. The yolk globules become enlarged because of their fusion with one another. There is further increase in the weight and volume of the ovaries.

Pre-spawning phase (April to May) Plate I e,f : This stage extends from April to May. During this phase the ovaries become deep yellow in colour. Vascularisation is extensively developed. Both transparent and opaque ova are present and the ovaries attain maximum weight. The fish abdomen seems bulging due to the presence of ripe ova inside. In this phase nuclear extrusion is seen. Histological condition is the same as that of previous months.

Spawning phase (early June to late July) Plate 1f,Plate-3m : This phase begins in the early June and ends in the late July. During this period the ovaries are yellowish and turgid due to the presence of a large number of translucent eggs. Ripe eggs are present in the oviduct to be discharged out side. The fish spawns number of times during this period. Vascularisation of the ovary reaches its peak and the ovaries are said to be in running phase. In this phase ova ooze out in the oviduct with slight pressure in the abdomen. Tunica albuginea becomes extremely thin and inter follicular space is greatly reduced. The ovigerous lamellae are inconspicuous. Histological section of the ovaries in June and July shows a number of discharged follicles, such follicles can be detected up to the end of September.

Spent phase (August to September) :This phase starts in August and lasts up to September. The ovaries become thin, flaccid, delicate, slender and dull in colour. There is a decrease in the volume and weight of the ovary.

Vascularisation is reduced. In this phase, oogonia are seen budding from the germinal epithelium. The tunica albuginea again becomes thicker. Histologically, the ovary shows some residual oocytes as well as discharged follicles. The nest of oogonia are seen among the ovigerous lamellae.

5. Discussion

Xenentodon cancila (Ham.) does not show sexual diamorphism, but during the breeding period, they exhibit notable sexual difference by the presence of relatively enlarged belly in female fishes. In this fish, the ovary is of cystovarian type, because lumen of the ovary is continuous with the oviduct.

5.1 Origin of Oogonia

Different authors have expressed different views regarding the origin of the new oogonia, Yamamoto [18] believed that the new oogonia originate from the follicular epithelial cells of spent follicles but according to, [,19,20] the new crop of the oogonia are produced by the germinal epithelium.Unlikely to the above suggestion some [7-9] suggest that in *Schizothorax richardsonii* the new crop of oogonia arises from the residual oogonia. As observed by Rita and Nair [13] the new crop of oogonia originates from ovarian lamellae formed by germinal epithelium. Shrestha and Khanna [12] showed that the crop of oogonia arises from the residual oogonia. In this fish*, cancila* the crop of oogonia are derived from the residual oogonia. This finding supports the finding of Belsare [4,8,12].

5.2 **Yolk Nucleus**

The investigators differ in their views regarding the origin and function of the yolk nucleus. According to Yamamoto [21] the yolk nucleus is of nuclear in origin, while Chopra [22] believed it to be cytoplasm in origin. Wallace [17] suggested that the yolk nucleus has the capacity to form yolk in fishes, whereas Wheeler [23] suggested that the yolk nucleus remains inactive and takes no visible part in the formation of yolk. Khanna [24] has suggested that the yolk nucleus is the initial centre of growth and dispersal of all important cellular inclusions. Bara [20] and Nayar [25]. reported some relationship between the yolk nucleus, golgi bodies and mitochondrial elements According to Rita and Nair [13] the yolk nucleus is entirely wanting in any stage of oocyte maturation of *Lepidocephalus thermalis*.

In *X. cancila* the yolk nucleus is present in the periphery of young stage III oocytes. As it gradually disappears in the maturing oocytes, it may have some relationship with the process of vitellogenesis.A similar view has been expessed by Khanna, and Sanwal [8] in *Channa gachua* and Bisht and Joshi [4] in *Schizothorax richardsonii*

Plate-1

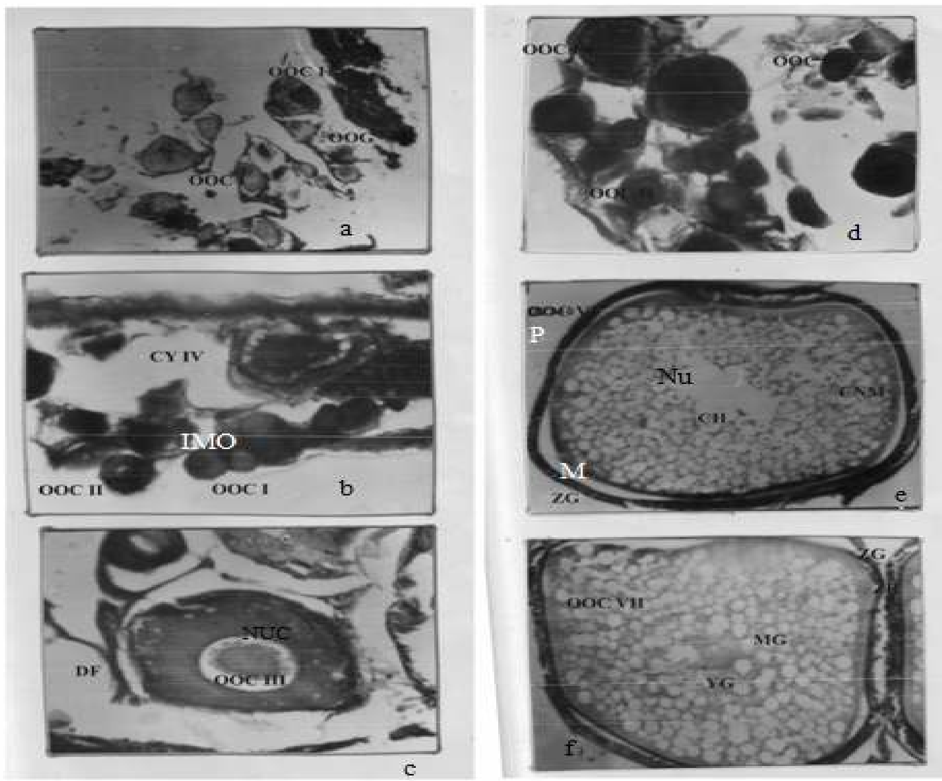

Plate – 2

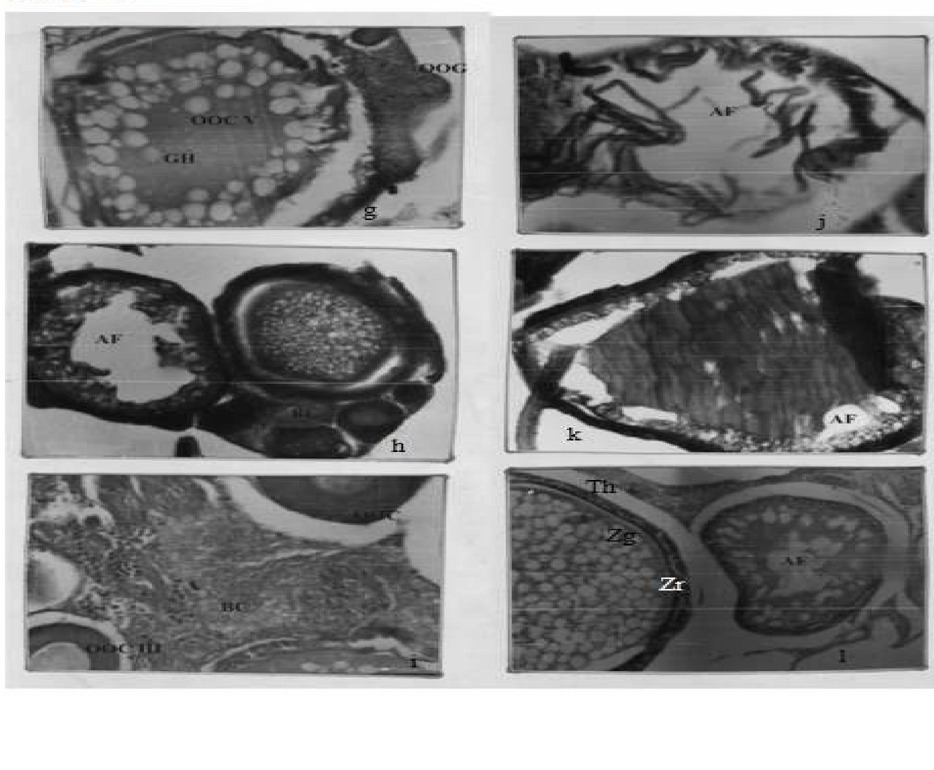

Plate- 3

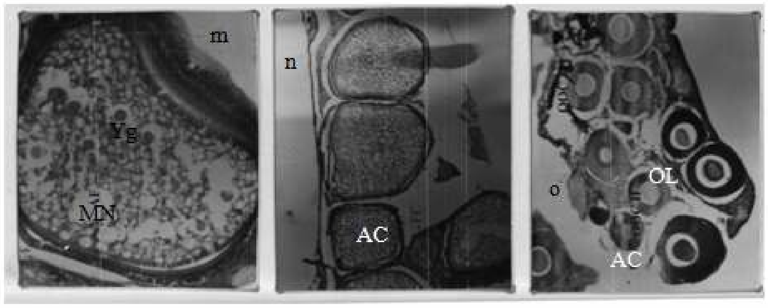

Plate -1 a. Photomicrograph of T.S. ovary of *Xenentodon cancila* showing oogonium,ooc l, x 40

b. General observation of T.S. ovary of *X.cancila* showing stage 11 and IV,ooc l x 100.
c. General observation of T.S. ovary of *X.cancila* showing stage III and yolk nucleus x 200
d. T.S. ovary of *X.cancila* showing appearance of yolk vesicles in the periphery in stage V&lll oocytes x 200.
e. T.S. ovary of *Xenentodon cancila* showing stage Vl x 200
f. T.S. ovary of *Xenentodon cancila* showing stage Vll x 200

(GE = Germinal epithelium, 10 = Immature oocyte, MN= Migratory nucleus, Ns = Nest, Nu = Nucleus, NM = Nuclear membrane, 0 = Oogonium, OOC = Oocyte, VM= Vitelline membrane, YV = Yolk vesicle, YG = Yolk granule)

Plate -2 g. T.S. mature oocyte of *Xenentodon cancila.* showing stage IV x 50.

h. Showing fusion of yolk globules x 100
i. Stage VII showing migratory nucleus x 100.
j. Plate of a ripe ovum x 200.
k. Showing both mature and immature oocytes.
l T.S. ovary of *X.cancila* showing immature oocytes. ovocoel.

(FL = Follicular layer, 10 = Immature oocyte. MN = Migratory nucleus, MO = Mature oocyte, NU = Nucleus,. TH = Thica, VM = Vitelline membrane, YG = Yolk globules, YV = Yolk vesicles; ZR = Zona radiata.)

Plate-3 m. T.S. ovary of *Xenentodon cancila* showing stage Vll x 200
YG=yolk globules,MN=migrating nucleus,YV=yolk vesicles ,

n. TS showing of ovary of *X.cancila* atretic egg
o. TS of ovay of *X.cancila* showing P= Peritoneum,BC-Blood capillary,GE=Germinal epithelium,IO=Immature oocyte, AC = Atretic egg

5.3 Nuclear Extrusion

There are several views regarding the origin, extrusion and functions of the nucleoli in teleosts. Rai [6] believed that large number of nucleoli are produced by division and fragmentation of a single nucleus of an oogonium, contrary to the above view, Yamamoto et.al. [8,13,18,20,] did not observe any fragmentation in the fishes examined by them. According to their views, the fusion of minute fulgen positive particles present in peripheral nucleoplasm form peripheral nucleoli. According to Bisht [5,9 23] in *Schizothorax richardsonii* the nucleoli are produced by the division and fragmentation of the nucleolus.

In *X.cancila* many nucleoli of different sizes were observed in the early stages of oocyte, increasing in number by division, but the number diminishes as oocyte advances to maturity. This result corroborates closely with the finding of Khanna *et. al*.[5,8,23,]. Extraction of nucleoli has been studied by several investigators in the past, but there is a great controversy in the origin of extrusion of nucleoli. According to Dixit [3] the nuclear membrane does not allow the nucleoli to pass through it. Rai et. al [6 ,8] have also observed extrusion of nucleoli into the ooplasm. Extrusion of nucleoli is seen in *X. cancila.* Several nucleoli of various sizes are seen in the early stage of oocyte. As oocytes reach maturity the nucleoli are seen increasing in number and their size goes on diminishing. This result agrees with the finding of Khanna [5]. As the extrusion of nucleoli was seen more in number during the formation of yolk. It appeared to be associated with the formation of yolk.

5.4 Yolk Formation

According to the mode of yolk formation the fish eggs have been classified into two types by Yamamoto [26] Uamamoto namely (i) eggs having a continuous mass of yolk, as in *Oryzia* [18] and (ii) eggs having a noncontinuous mass of yolk, as in *Clupea* [27]. In *X.cancila* yolk appears at the periphery of oocytes, in the form of the globular, later the whole ooplasm is filled with yolk globules. This process is similar to *Clupea* [21] *Tot tor* [6] *Channa gachua* [8], *Puntius sophore* [28]

Similarly results were obtained in *X.cancila*, where the yolk vesicles and yolk globules are independent of each other and the former give rise to so called cortical alveoli. And the yolk vesicles as such seen to play no role in the formation of yolk globules. Nayar et. al [21,25] have described breaking down the vesicles of the inner side to take place in the formation of yolk globules in *Cyprinus* and *Gobio* which contradicts the above findings.

5.5 Spawning

There are three modes of oocytes development, according to Prabhu [29] in some fishes eg. *Oncorhynchus masou* [20] all the oocytes develop together at the same time in the ovary, such fishes spawn only once in a life time. This phenomenon is called total synchromism, there are some species which spawn once or twice in a season, developing two group of oocytes side by side (group synchronism), as in speckled trout, Viadykov [30] still other species spawn several times in a breeding season. In these species large number of oocytes are present at various stages of their development in the same ovary asynchronism) as in *Channa gachua* [8] and *Puntious sophore* [28].

The spawning season of *X.cancila* extends from early June to late July. Since various stages of developing oocytes are found in the same ovary and the fish spawns several times during a breeding season. *X. cancila* comes under the third group asynchronism.

References

[1] A .L. Craig-Bennett,Linn. Philos. Trans. B 219 (1930)197.
[2] C.F. Hickling, Z.J. Mar. Biol, Ass. Y.K. 20 (1936)443.
[3] R.K. Dixit, Proc. Nat. Acad, Sci, India B. 30 (1956) 214.
[4] D.K.Belsare, Indian J.Fish. 9 (1962)140.
[5] S.S. Khanna, and Pant, M.C. Copeil (1967) 83.
[6] B.P.Rai,Acta.Zool. 48 (1967)289.
[7] G.K. Lehri, Acta Anat. 69 (1968) 105.
[8] S.S. Khanna, and R. Sanwal, Zool. Geitr, Floge, 17 (1971)311.
[9] J.S.Bisht and M.L.Joshi, Acta, Anat. 93 (1975)512.
[10] C.P Kapoor,Acta. Anat, 96 (1976)1-8.
[11] C.P. Kapoor, Zekrosk. Anat, Forach. (Leipz) 90 (1978)1049.
[12] T.K. Shrestha and S.S. Khanna, Matsya, 5 (1979)23.
[13] S.D. Kumari Rita and N.B. Nair, Acad. Sci. Sect.B. 88 (1979)45.

[14] K. Toshimori, and Y. Fumioki, Forsch (Lepiz) 93 (1979) 458.
[15] P.Chakrabarti and D.K. Mandal,Journal of Freshwater Bil.7 (1995)195.
[16] B.R Subba,J. Freshwater Biol.10 (1998)103.
[17] W. Wallace, Quart. J. Micr. Sci. 47 (1904)161.
[18] K.Yamamoto,Ann.Zool.Jap.28 (1955)233.
[19] V.Sriramalu and M. Rajalaxmi, Z. Mikrosk. Anat, Forsch., 75 (1966)65.
[20] G.Bara, Rev.Fac,Sci, Univ. 25 (1960)50.
[21] K.Yamamoto, H.Kal and R.Ished,20 (1959)109.
[22] H.C. Chopra, Experimentia. 17 (1961)120.
[23] J.F.C. Wheeler, Quart. J. Micr. Sci. 68 (1925)641.
[24] H.S. Chaudhary, Z.Zellforosch. 37 (1952)455.
[25] R.P.Nayar, Quart. J. Micr, Sci. 105 (1964)353.
[26] K.Yamamoto, J.Fac.Sci.Hokkaido Univ.13 (1957)346.
[27] K.Yamamoto,Bull.Fac.Fish.Hokkaido,Univ.8 (1958)270.
[28] R.K.Dixit and N.Agrawal,Acta.Anat.90 (1974)133.
[29] M.S.Prabhu, Indian J.Fish 3 (1956)59.
[30] V.D.Viadykov, J. Fish. Res. Canada 13 (1956)799.